IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Yechezkel BARENHOLZ et al. Appln. No.: 09/780,757 Filed: August 2, 2001 For: DETECTION OF BINDING OF CHARCED SPECIES USING PH OR POTENTIAL SENSITIVE PROBES	ATTY.'S DOCKET: BARENHOLZ=1 Art Unit: 1637 Examiner: FREDMAN, Jeffrey Norman Washington, D.C. Confirmation No. 6619

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window
Randolph Building, Mail Stop
401 Dulany Street
Alexandria, VA 22314

Sir:

I, Yechezkel BARENHOLZ, do hereby state and declare as follows:

I am an inventor of the above-identified application and my education and professional experience was provided in the CV attached to my Declaration **filed June 22, 2005.**

The present invention describes methods for detecting binding of species to a given surface having a defined pH or surface potential by the use of a probe which comprises a pH and/or potential sensitive fluorophore.

In one aspect of the invention the pH and/or potential sensitive fluorophore is covalently attached to a surface and a change in a fluorescence is observed upon

binding (or dissociation) of the species at the surface due to a change in surface potential and/or in pH. The change in fluorescence thereby serves as an indicator for the association or dissociation between the surface and the species.

In a recent experiment a FITC-albumin (0.15mmol in PBS, pH 10) was used as pH or potential sensitive surface[s]. The fluorescence intensity of the FITC-labeled albumin surface was measured before (-) and after (+) the addition of cationic liposomes (DOTAP) or cationic polymers such as polyethyleneimine (PEI) or polylysine.

As shown in the following Tables A-C a change in fluorescence intensity occurred when the FITC-labeled albumin surface was brought into contact with the cationic liposomes (DOTAP) as well as when brought into contact with the cationic polymers (polyethyleneimine (PEI) and polylysine).

No change occurred when negatively charged (PC/PG) liposomes were used (Table D).

Table A - Detection of binding of DOTAP

DOTAP (0.1nmol)	Fluorescence intensity*
-	195
+	154

* Fluorescence intensity in arbitrary units as measured at an excitation wavelength of 495nm and an emission wavelength of 520nm with slits of 2.5 nm and 2.5nm respectively.

Table B – Detection of binding of polylysine

Polylysine (25µg)	Fluorescence intensity*
–	154
+	68

* Fluorescence intensity in arbitrary units as measured at an excitation wavelength of 495nm and an emission wavelength of 520nm with slits of 2.5 nm and 2.5nm respectively.

Table C – Detection of binding of polyethyleneimine**

Polyethyleneimine (30µg)	Fluorescence intensity*
–	424
+	389

* Fluorescence intensity in arbitrary units as measured at an excitation wavelength of 495nm and an emission wavelength of 520nm with slits of 5nm.

Table D – Detection of binding of PC/PG**

PC/PG (0.1nmol)	Fluorescence intensity*
–	169
+	176.8

* Fluorescence intensity in arbitrary units as measured at an excitation wavelength of 495nm and an emission wavelength of 520nm with slits of 2.5 nm and 2.5nm respectively;

**PC/PG – Phosphatidylcholine/Phosphatidyglycerol.

The experiment reported above was conducted under my supervision and thus I have first hand knowledge of the results thereof.

The results show that when the species to be detected are added to FITC-albumin, the fluorescence intensity is decreased. Thus, these results provide further evidence that the method of the invention is applicable for a variety of polymers.

I herby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 81 of the united states code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon;

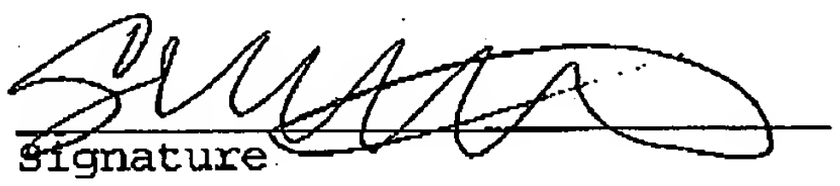
Yechezkel BARENHOLZ

August 14. 2005
Date

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being facsimile transmitted to Examiner Jeffrey Fredman at 703-872-9306 of the Patent and Trademark Office on the date shown below.

Sharnita Davenport
Name



Signature

August 15, 2005
Date